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Measurement of Prostate Specific Antigen Using Self-Sensing Nanomechanical Membrane

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Abstract

In this work, a signal transduction biosensor has been used as a novel electrical detection for identifying prostate specific antigen (PSA), a protein biomarker associated with prostate cancer. A direct nano-mechanical response of micro-fabricated self-sensing nanomechanical membrane (NMM) was used to detect the surface stress changes of antigen–antibody specific binding. The sensing principle is based on the surface stress changes induced by antigen–antibody interaction on the NMM surfaces. NMM consists of a membrane suspended by four piezoresistive sensing components. The isotropic surface stress on the membrane results in an uniaxial stress in each sensing component, which efficiently improves the sensitivity. After injecting the PSA target, as model biocontents, the piezoresistive responses were carefully analyzed and the feasibility of the piezoresistive membranes for biosensing were discussed in terms of device performance measures such as sensitivity, accuracy, and specificity. At the end, the results were compared with a standard cantilever.

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1. Experimental

In this study, we have used arrays of NMM to detect PSA. A simple illustration of the final NMM sensor with

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piezoresistive sensing component can be observed in figure 1a.. The membrane-type geometry allows us to place a full Wheatstone bridge on the chip, when all four resistors are practically equal and the relative resistance changes are small, the total output signal V_{out} can be approximated by:

$$V_{out} = \frac{V_{in}}{4}\left(\frac{\Delta R_1}{R_1} - \frac{\Delta R_2}{R_2} + \frac{\Delta R_3}{R_3} - \frac{\Delta R_4}{R_4}\right) \quad (1)$$

According to equation, the average value of relative resistance change in the NMM has a higher value in comparison with the standard cantilever (about 43 times)[1].

1.1. Fabrication of NMM sensor

We used Silicon on Insulator (SOI) wafers with a 2 μm device layer and a 0.3 μm buried oxide (BOX) layer as the substrate material. Then a 25 nm silicon dioxide layer was grown by a thermal oxidation to electrically insulate the device layer from the subsequent metal layers. The first lithographic process to define the first metal layer for electrode and sensor platform for subsequent liftoff process has been accomplished. After patterning, the photoresist, chrome (10 nm) and gold (50 nm) layers were deposited by e-beam evaporator and patterned by a liftoff process with the previously patterned photoresist. The patterned metal layer from previous step and the patterned layer of photoresist, from the second photolithographic process were used to define the areas to be etched to define the sensor structure. The exposed device layer was etched completely by RIE to define the sensor structure. Then, a third photolithographic step for the second liftoff process, followed by the deposition of a 30-nm chrome layer and a 150-nm gold layer for wire-bonding pads. After the liftoff, a release window was photolithographically defined by the fourth lithographic process and the exposed BOX was etched by RIE leaving the Si substrate exposed. Then the wafer was diced into individual chips. Through the release window, the exposed Si substrate was etched by vapor phase etching using xenon difluoride (XeF_2) to release the sensor structure. After XeF_2 etching, the photoresist and the BOX were removed by BHF etching and solvent cleaning. The die was cleaned with oxygen plasma and then a 100-nm thick silicon dioxide layer was deposited with plasma enhanced chemical vapor deposition (PECVD) for insulation. Chrome (20 nm) and gold (50 nm) layers were deposited using an e-beam evaporator for an immobilization layer for protein–protein interaction. The PECVD oxide on the bonding pads was selectively etched for wire-bonding. Then each die was attached to a custom made printed circuit board (PCB) and was wire-bonded. Fig. 1b presents the final picture of NMMsin different sizes fabricated in the same array using a Scanning electron micrograph (SEM).

1.2. PSA antibody immobilization process

A fresh piranha solution (a 4:1 ratio of H_2SO_4 (98.08%) and H_2O_2 (34.01%)) was used to wash and clean the membranes, in order to remove experimental contamination of the Au surface. After 1 min, the membranes were taken out of the solution and were rinsed using deionized water. To complete the cleaning process, the rinsed membranes were dried using a stream of N_2 gas. For 2 h at room temperature in darkness a 0.1 M deoxygenated cysteamine (Sigma, 95%) aqueous solution was used to functionalize the devices. Then, NMMs were washed with deionized water and soaked in water for 12 h to remove the physically adsorbed cysteamine. Moreover, for creating a covalent cross-linker molecule between the amine groups on the NMM surface and antibodies, chips were soaked in a 5% solution of gluteraldehyde (Sigma, 50%) in borate buffer for 2 hours. Following this and all subsequent steps, device chips were washed twice, each washing step was for two minutes, in purified DI water on an orbital shaker operating at 95 RPM. It should be mentioned that fresh water was used between washes. The reason of using water instead of buffer for washing was to prevent the abundant formation of buffer salt crystals on the surface of devices which make the sensors effectively useless.

Next, one hour incubation was used to immobilize the monoclonal anti-PSA (anti-prostate specific antigen, Fitzgerald Industries International Inc., Concord, MA, USA), affinity-purified, with a concentration of 50 mg/mL on

the surface. By immersing the NMM in 50 mM solution of glycine for 30 minutes unreacted gluteraldehyde was then quenched. In addition, dissolved bovine serum albumin (BSA, Sigma, St. Louis, MO, USA) in phosphate buffered saline (PBS) with 10 mg/ml concentration was used to prevent non-specific binding. For this purpose, the membranes were immersed in this solution for 1 h at room temperature. Then, they were rinsed with PBS (pH 7.4) containing polyoxyethyethylenesorbitan monolaurate (Tween 20, St. Louis, MO, USA) and finally washing was performed by only using PBS solution.

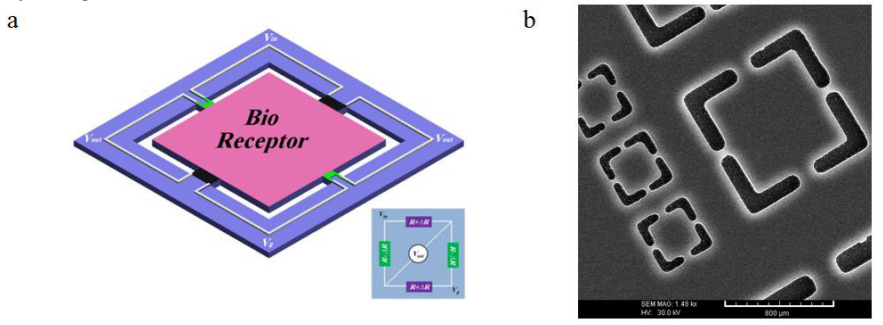

Fig. 1. (a) A schematic of the NMM sensor with piezoresistive sensing component; (b) Scanning electron micrograph (SEM) of NMM array chip with two different dimension of 800 µm x 800 µm x 2 µm and 400 µm x 400 µm x 2 µm which fabricated in a same array.

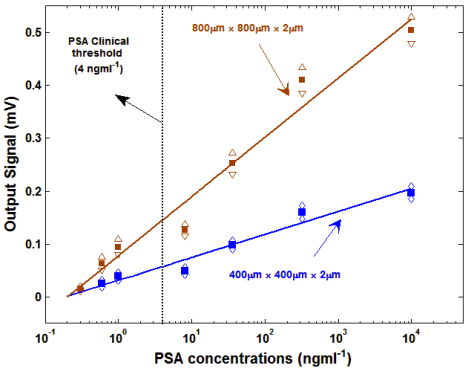

Fig. 2. Steady-state output signals (V_{out}) as a function of PSA concentrations for two different NMM geometries. Every data point on this plot represents an average of output signals obtained in multiple experiments done with different NMM, whereas the range of output signals obtained from these experiments is shown as the error bar.

2. Results and discussion

In order to reach results with high reliability, the surfaces of the membranes were stabilized by treating them with a PBS buffer. The PBS buffer was directed with a typical flow rate of 0.4 – 0.5 ml/hour, for 1 h, to the NMM sensor arrays using a flexible PDMS polymer microfluidic channel sealed to the device chip. As a general trend, at the point of initial injection of the PBS buffer the induced voltage of the NMM increased rapidly and steadily decreased with time, which in this case the induced voltage of the NMM reached dynamic equilibrium after 10 min. For the bio-assay, PSA antigens were injected into each liquid chamber, including the stabilized membrane. The liquid temperature was precisely controlled and external noise sources were excluded using a shield box. In order to estimate the nonspecific adsorption on the NMM surface, the concentration of BSA in all solutions was stabilized at 0.1 mg/ml. Figure 2 shows the steady-state output signals (V_{out}) as a function of PSA concentration in a BSA background for different dimensions of NMM. By using a 400 µm x 400 µm x 2 µm NMM, the lowest PSA

concentration that we could clearly detect above noise was 0.6 ng/ml. However, when a 800 µm x 800 µm x 2 µm NMM was used, PSA concentration as low as 0.3 ng/ml was detectable. This is close to the resolution required for PSA-based diagnosis of prostate cancer [2]. The experimental results presented a range of linearity of 0.3 ng/ mL to 10 µg/mL and 0.6 ng/mL to 10 µg/mL for 800 µm x 800 µm x 2 µm and 400 µm x 400 µm x 2 µm NMM, respectively. The minimum detectable surface stress for each sensor can be obtain when the output signals are equal to the noise values. By using the experimental results, 1.5 and 2.5 mJ/m were respectively the minimum surface stress sensitivities for the 800 µm x 800 µm x 2 µm and 400 µm x 400 µm x 2 µm NMM.

In order to check the sensitivity of the present NMM -based biosensor, the results have been compared with other label free biosensing technologys. In Table 1, the minimum PSA detection limits (LOD) of NMMs with different dimensions have been compared with a standard cantilever (MCL) [3 and 4], surface-plasmon resonance (SPR) [5], quartz crystal monitor (QCM) [6], and electrochemical [7] sensors. In most cases the NMM -based biosensor has the lowest LOD. Results indicat that NMM has comparable sensitivity with the optical read-out methods, moreover its sensitivity is significantely higher than a standard piezoresistive cantilever. This table quite well reflects the potential of the NMM -based biosensor in the pharmaceutical and medical diagnosis fields.

Table 1. Tables must be placed in the second page. Details can be outlined briefly in the captions.

Category	Detection conditions	LOD
NMM	0.1 mg/ml BSA	0.6 ng/ml [(a)]
	0.1 mg/ml BSA	0.3 ng/ml [(b)]
MCL [3] with reference cantilever, piezoresistive detection	0.1 mg/ml BSA	10 ng/ml
MCL [4] no reference cantilever, optical detection	1.0 mg/ml HSA	0.2 ng/ml
SPR [5] Direct immunoassay	0.3 mg/ml BSA	300 ng/ml
QCM [6] Direct assay based on yeast cells strategy	serum	5 ng/ml
Electrochemical [7] Amperometric Sandwich immunoassay	phosphate buffer	0.25 ng/ml

[(a)] 400µm x 400µm x 2µm.

[(b)] 800µm x 800µm x 2µm.

3. Conclusion

We have reported a novel signal transduction biosensor for detecting PSA, using a unique micro-fabricated self-sensing array of NMM sensors. Unlike cantilever sensors, which are based on optical readout systems, the NMM integrated piezoresistive readout sensors facilitate the detection of compact devices in even non-transparent environments. In comparison with traditional piezoresistive based cantilever sensors, our unique NMM design significantly improves sensor sensitivity that allows us to detect PSA concentrations as low as 300 pg/mL.

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